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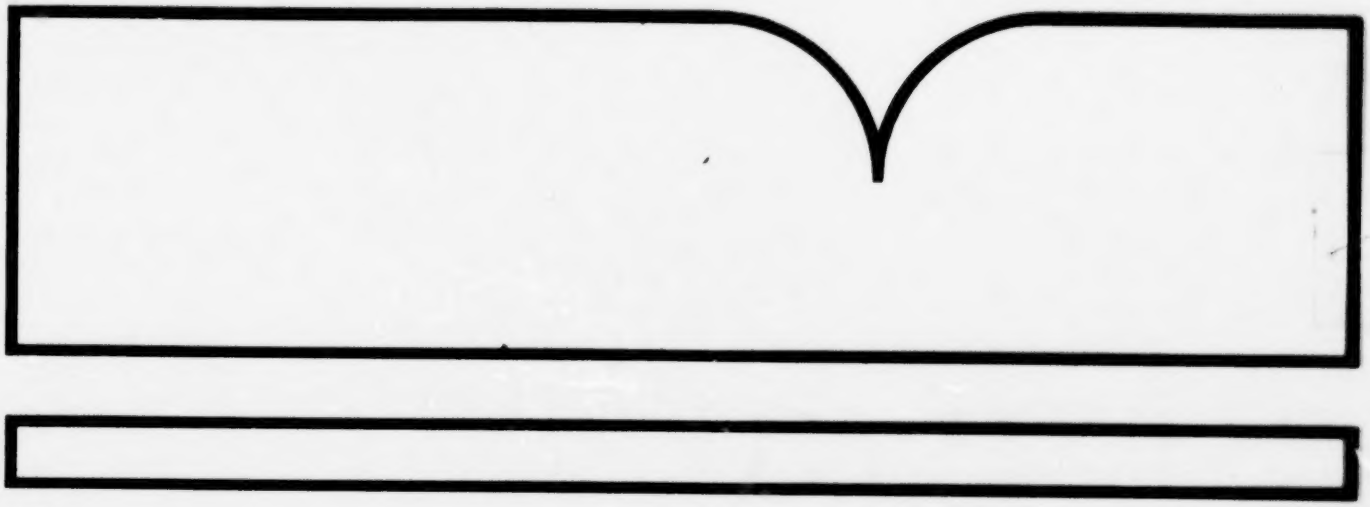

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16. ABSTRACT

Acute (96-hr) flow-through toxicity tests, chronic (entire life-cycle) tests, and bioconcentration studies were conducted on selected estuarine animals with the insecticides, EPN, and leptophos. In the EPN acute toxicity tests, the test animals and their 96-hr LC50 values were: Mysidopsis bahia, 3.44 µg/1; Penaeus duorarum, 0.29 µg/1; Cyprinodon variegatus, 188.9 µg/1; Lagodon rhomboides, 18.3 µg/1; and Leiostimus xanthurus, 25.6 µg/1. Test animals and 96-h LC-50 values for leptophos were M. bahia, 3.16 µg/1; P. duorarum, 1.88 µg/1; and L. xanthurus, 4.06 µg/1. In separate chronic tests, M. bahia were exposed to EPN and leptophos. Significant (α=0.05) mortality and fewer young were produced in 4.13 µg/1 EPN. In the leptophos chronic test, significant mortality occured in concentrations $\geq$3.63 µg/1 and fewer young were produced in concentrations $\geq$1.77 µg/1. L. rhomboides, exposed to EPN in a 26-day uptake study, bioconcentrated the insecticide 707 X that measured in the exposed water. Our results indicate that if either contaminated an estuarine environment in concentrations $\geq$1.0 µg/1, the most profound adverse effects on crustaceans and possibly fishes would result from acute toxicity, rather than from chronic toxicity or bioconcentration of the chemicals.

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TOXICITY AND BIOCONCENTRATION OF EPN AND LEPTOPHOS TO SELECTED ESTUARINE ANIMALS[1]

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ABSTRACT

Acute (96-hr) flow-through toxicity tests, chronic (entire life-cycle) tests, and bioconcentration studies were conducted on selected estuarine animals with the insecticides, EPN, and leptophos. In the EPN acute toxicity tests, the test animals and their 96-h LC50 values were: *Mysidopsis bahia*, 3.44 μg/l; *Penaeus duorarum*, 0.29 μg/l; *Cyprinodon variegatus*, 188.9 μg/l; *Lagodon rhomboides*, 18.3 μg/l; and *Leiostomus xanthurus*, 25.6 μg/l. Test animals and 96-h LC50 values for leptophos were: *M. bahia*, 3.16 μg/l; *P. duorarum*, 1.88 μg/l; and *L. xanthurus*, 4.06 μg/l. In separate chronic tests, *M. bahia* were exposed to EPN and leptophos. Significant ($\alpha = 0.05$) mortality and fewer young were produced in 4.13 μg/l EPN. In the leptophos chronic test, significant mortality occurred in concentrations $\geq$ 3.63 μg/l and fewer young were produced in concentrations $\geq$ 1.77 μg/l. *L. rhomboides*, exposed to EPN in a 26-day uptake study, bioconcentrated the insecticide 707 X that measured in the exposure water. When *L. rhomboides* were held in EPN-free seawater, no EPN was detected in their tissues after eight days. *L. xanthurus*, exposed to leptophos for 26 days, bioconcentrated the insecticide to only 68 × the concentration in the exposure water. No leptophos residues were detectable in tissues after 4 days depuration. Our results indicate that if either insecticide contaminated an estuarine environment in concentrations $\geq$ 1.0 μg/l, the most profound adverse effects on crustaceans and possibly fishes would result from acute toxicity, rather than from chronic toxicity or bioconcentration of the chemicals.

INTRODUCTION

In April 1977, the Environmental Research Laboratory, Gulf Breeze, Florida, was requested by the Environmental Protection Agency's Region VI to conduct toxicity and bioconcentration studies on the insecticides EPN and leptophos. The request was prompted by the detection of these chemicals in water, sediment, and biota adjacent to the Velsicol Chemical Company's Bayport Plant, Texas, and in oysters of nearby Galveston Bay.

Leptophos (Phosvel®; 4-bromo-2,5-dichlorophenyl phenylphosphonothioate) is manufactured in the United States and exported but not registered for use on crops in the U.S. except under provisions of a temporary permit on limited acreage.

The toxicity to or bioconcentration of leptophos by aquatic biota has been de-

[1] Gulf Breeze Contribution No. 354.

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scribed in few published studies. In a 45-day study (Metcalf and Sanborn 1975), the fate of leptophos was determined in a model terrestrial-aquatic ecosystem. Results indicated that leptophos was accumulated 2 to 550 times greater in the fish and snails of the ecosystem than ten other organophosphate insecticides tested.

EPN (O-ethyl-o-p-nitrophenyl phenyl phosphonothioate) is registered for use on agricultural crops in the United States, and its acute toxicity to several freshwater organisms has been studied. The 96-h LC50 values reported for various species of freshwater fishes exposed to EPN ranged from 32 μg/l to 450 μg/l (Pickering *et al.* 1962); those for several freshwater invertebrates ranged from 0.06 μg/l to 7 μg/l (Crosby *et al.* 1966; Sanders 1972).

Acute toxicity data from studies on estuarine species were similar. The range in acute LC50 values for fishes was 60 μg/l (96-h) to 320 μg/l (48-h) (Korn and Earnest 1974; Schoettger 1970; and J. I. Lowe, Environmental Research Laboratory, Gulf Breeze, Florida, personal communication); the 48-h LC50 for the commercial pink shrimp (*Penaeus duorarum*) was 3.2 μg/l (J. I. Lowe, personal communication).

No LC50 value described above was based on flow-through tests and measured concentrations of EPN or leptophos in seawater. No study described the chronic toxicity of either insecticide to estuarine animals or the accumulation of the insecticides in the animals' tissues. Our research sought to determine: (1) the acute toxicity of EPN and leptophos to several estuarine species based on measured concentrations of the insecticides in flowing seawater; (2) the accumulation of the insecticides in flowing seawater; (2) the accumulation of the insecticides in tissues of animals surviving a 96-h test; (3) the chronic (entire life cycle) toxicity of leptophos and EPN to the estuarine mysid, *Mysidopsis bahia*; and (4) the uptake rates, depuration rates, and bioconcentration factors of EPN using pinfish (*Lagodon rhomboides*) and leptophos using spot (*Leiostomus xanthurus*).

METHODS AND MATERIALS

Test Animals

All test animals, except mysid shrimp (*M. bahia*), used in 96-h toxicity tests, were collected in estuarine waters near the Environmental Research Laboratory, Gulf Breeze, Florida. Mysids were cultured in the laboratory following the methods of Nimmo *et al.* (1977). The animals were used in the toxicity test if mortality was less than 1% in the preceding 48 h and if they exhibited no obvious diseases or abnormal behavior during acclimation. No animals were fed during toxicity tests except the mysid shrimp that were fed brine shrimp (*Artemia salina*) nauplii *ad libitum*.

Juvenile pinfish and spot were used in separate EPN and leptophos bioconcentration studies, respectively. The fish were collected from estuarine areas near the laboratory and acclimated to laboratory conditions at least 14 days prior to testing. Criteria used to determine acceptable test animals were the same as those for the acute toxicity tests. Fishes were fed frozen adult brine shrimp at 10% body weight per day; no EPN (< 0.02 mg/kg) or leptophos (< 0.025 mg/kg) was detected in brine shrimp by electron-capture gas chromatography.

Mysid shrimp, used for the 21- or 28-day entire life-cycle tests, were produced from laboratory cultures. Mysids were fed live brine shrimp nauplii *ad libitum* each day.

Acute Toxicity Tests

Methods used in the acute toxicity tests, except in the case of mysids, followed the flow-through toxicity test procedures of the American Public Health Association (1975). The only modification of methods was the feeding of brine shrimp nauplii to mysids to prevent starvation during the 96-h period. The acute toxicity of EPN or leptophos was determined by exposing 20 animals per aquarium to each concentration for 96 h. Analytical grade EPN (97.2%) or leptophos (98%) was dissolved in triethylene glycol (TEG) and metered by pumps at 10 ml per day into the filtered seawater that entered each aquarium from siphons calibrated to deliver 30 l/h. One control aquarium received the same quantity of seawater and solvent with no insecticide; the second received only seawater at 30 l/h. Mortality was recorded daily, and dead animals were removed when discovered. Fishes and pink shrimp surviving the tests were rinsed with acetone and analyzed for whole-body residues.

Toxicity tests with mysids, because of their small size, required several modifications in the above procedures. We placed five animals (24-h-old juveniles) in each of four replicate chambers (10 cm × 15 cm) in each test concentration, using the methods of Nimmo *et al.* (1977). Seawater flow for the leptophos studies was delivered by a diluter at the rate of 3.7 l/h. Surviving animals were not analyzed for residues.

Mysid Entire Life-Cycle Studies

Mysid shrimp were exposed to EPN or leptophos in two separate studies, using the methods of Nimmo *et al.* (1977). The leptophos test was conducted using a diluter (Mount and Brungs 1967); the EPN test, syringe pumps and calibrated siphons (Nimmo *et al.* 1977). Two controls were provided for the EPN test (seawater with and without the carrier, TEG, at 0.05 ml/l). In the leptophos study, control seawater with 0.05 ml/l TEG was provided. In the EPN study, nominal concentrations were : 3.0, 0.3, 0.1, and 0.075 µg/l. Those for the leptophos study were: 5.0, 2.5, 1.3 and 0.63 µg/l. As each aquarium achieved maximum volume, a self-starting syphon drained the water to a volume of approximately one liter. Fluctuating levels of seawater, occurring at about 30 minute intervals, ensured an exchange of seawater within each aquarium and small retaining chambers (a Petri dish bottom to which a 15 cm high nylon mesh collar was attached). A small stream of compressed air was delivered into each chamber to safeguard against possible anoxic conditions and to create a current that apparently aids in the orientation of the test animals.

Approximately forty 48-h old juveniles (4 or 5 per chamber) were exposed to each concentration of EPN or leptophos for a period of 28 to 21 days, respectively. During this exposure period, juvenile mysids mature and produce at least one brood. Number of live animals by sex, number of females with and without brood pouches, and number of young were recorded daily; dead animals were removed when observed.

Fish Bioconcentration Studies

Juvenile pinfish (44–77 mm standard length; $\bar{x} = 59$ mm) were exposed to EPN or 26 days followed by an 8-day depuration period. The exposure apparatus was the same used by Schimmel *et al.* (1977a). A stock solution of EPN dissolved in TEG was prepared at the beginning of the test and calculated deliveries were 0.25 and 2.5 µgEPN/l seawater. Each exposure concentration was duplicated; two control aquaria were provided, one with and one without TEG. We selected these concentrations because: (1) acute toxicity studies indicated that they were well below the EPN lethal threshold for pinfish; and (2) they were quantifiable in gas chromatographic analyses.

Thirty juvenile pinfish were placed in each experimental and control aquarium at the beginning of the test. Two fish from each control, 0.25 and 2.5 µg/l aquarium were sampled for bioconcentration at 24, 48, and 96 hours, and days 8, 15, 18 and 22. The chemical exposure was terminated after 26 days and remaining fish were held in EPN free seawater and sampled on days 1,2,4, and 8. Tissues were analyzed on a whole-body wet-weight basis. Seawater temperature averaged 25°C (range = 23 to 28°C); seawater salinity averaged 25.7 ‰ (range = 22 to 31 ‰).

Juvenile spot were exposed to leptophos for 26 days followed by a 4-day depuration period. The exposure apparatus (Schimmel *et al.* 1977a) was the same as that used in the EPN study. A stock solution of leptophos, dissolved in TEG, was prepared at the beginning of the test. Experimental concentrations delivered were 0.04 and 0.4 μg/l. Each exposure concentration was duplicated and two control aquaria were provided, one with and one without TEG. The concentrations selected were based on the same criteria as the EPN test.

Fifty juvenile spot were placed in each exposure aquarium at the beginning of the test. Three fish (pooled) from each control, 0.04 and 0.4 μg/l aquarium were sampled at 24 and 96 h, and twice each week thereafter for 26 days. After 26 days, the chemical exposure was terminated and remaining fish were held in leptophos free seawater. Each fish was dissected into edible tissues (muscle and skin without scales taken from each side of the fish above the lateral line) and remaining tissues (all portions minus the edible tissues). In the depuration period, fish were sampled at 24-, 48-, 72- and 96-h.

Statistical Methods

Shrimp and fish mortality data from acute toxicity tests were analyzed by using the probit analysis method of Finney (1971) to estimate concentrations of EPN and leptophos in water that killed 50% of the test animals (LC50), and the 95% confidence intervals for these concentrations.

In the mysid entire life-cycle studies, LC50 values (and 95% confidence intervals) for the 21- and 28-day exposures were calculated by the probit analysis of Finney (1971). We used Dunnett's test, comparing multiple treatments with control to mean brood size and analysis of variance with Student-Newman Keuls' *post hoc* test to determine significant differences in mortality of experimental and control mysids. Significant levels of 95% were used for all statistical calculations.

In the bioconcentration studies, the statistical model of Bahner and Oglesby (1978) was used to determine bioconcentration factors and to describe uptake and depuration of EPN and leptophos in pinfish and spot.

Chemical Methods

In the analysis of tissue samples for EPN and leptophos, one to eight grams of tissue were weighed into 150 mm × 25 mm (O.D.) screw-top test tubes and extracted four times with 5 ml portions of acetonitrile for 30 seconds using a model PT 10-ST Willems Polytron®. The test tube was centrifuged after each extraction and the acetonitrile transferred to a 120-ml oil sample bottle. To the combined extracts was added 75 ml of 2.0% aqueous sodium sulfate, and this solution was extracted with four 5 ml portions of hexane. The bottle was capped and shaken for one minute, the solvent phases allowed to separate, and the upper hexane layer pipetted into a 25 ml Kuderna-Danish concentrator tube. The combined extracts were then concentrated to 0.5 ml on a steam table with a micro Snyder column. The concentrate was quantitatively transferred with three 0.5 ml portions of hexane to a 9 mm Chromaflex column (Kontes Glass Company, Vineland, N.J.) containing 3.0 grams of Florisil topped with 2.0 grams anhydrous sodium sulfate that had been washed previously with 10 ml hexane.

For the analysis of EPN, the column was first eluted with 20 ml of 5% diethyl ether in hexane to remove PCB's and pesticides; EPN was then eluted with 25 ml of 10% isopropanol in iso-octane and the volume adjusted to the appropriate level for analysis by electron-capture gas chromatography.

For leptophos, the column was eluted with 20 ml of 5% diethyl ether/hexane solution and the volume was adjusted to an appropriate level for analysis by electron-capture gas chromatography.

One liter of seawater samples were fortified with heptachlorobiphenyl for EPN analysis and methyl parathion for leptophos analysis and extracted twice with 100 ml petroleum ether. The solvent extract was dried and concentrated to an appropriate volume on a steam table using a Kuderna-Danish concentrator.

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TABLE 1

Acute (96-hr) toxicity of EPN and lepthophos to several estuarine animals in flowing seawater tests.

INSECTICIDE	SPECIES	LENGTH[a] ($\bar{x}$,mm)	MEASURED CONCENTRATIONS LC50 in µg/l (95% confidence interval)	TEMPERATURE ($\bar{x}$, °C)	SALINITY ($\bar{x}$, °/oo)
EPN	Estuarine mysid *(Mysidopsis bahia)*	7.5	3.44 (2.49 - 5.84)	23.5	24.0
	Pink shrimp, *(Penaeus duorarum)*	69	0.29 (0.1 - 1.1)	25.0	22.0
	Sheepshead minnow, *(Cyprinodon variegatus)*	41	188.9 (150.0 - 255.2)	24.6	18.2
	Pinfish, *(Lagodon rhomboides)*	64	18.3 (14.7 - 23.5)	25.0	28.8
	Spot, *(Leiostomus xanthurus)*	51	25.6 (19.2 - 34.2)	24.0	23.2
LEPTOPHOS	Estuarine mysid *(Mysidopsis bahia)*	2	3.16 (1.89 - 5.67)	25.0	16.0
	Pink shrimp, *(Penaeus duorarum)*	---	1.88 (1.48 - 2.36)	27.1	27.2
	Spot, *(Leiostomus xanthurus)*	35	4.06 (3.65 - 4.71)	22.5	23.1

[a]Mysids, total length; pink shrimp, rostrum to telson length; fishes, standard length.

Analysis for EPN were performed on Hewlett-Packard Model 5730A electron-capture (^{63}Ni) gas chromatographs equipped with 182 cm × 2 mm (I.D.) glass columns packed with 2% SP 2100 on 100/120 mesh Supelcoport, 0.75% SP 2250; 0.97% SP 2401 on 100/120 mesh Supelcoport or 5% QF-1 on 80/100 mesh Gas Chrom Q. The operating parameters were: oven temperature 210°C; injector temperature, 200°C; detector temperature, 300°C; carrier gas, 10% methane/argon flowing at 25 ml/minute.

Analysis for leptophos in tissue and seawater were performed on a model 5710 Hewlett-Packard Gas Chromatograph with the columns and operating parameters as those used for EPN analyses.

Calculations for EPN and leptophos were made on a wet-weight basis without a correction factor for percentage recovery. The recovery values for EPN and leptophos from fortified tissues and seawater were greater than 85%.

RESULTS

Results of acute EPN and leptophos toxicity tests with mysids, shrimp and fishes are shown in Table 1. From these data, crustaceans are particularly sensitive to the two insecticides; pink shrimp were the most sensitive with 96-h LC50 values of 0.29 and 1.88 µg/l for EPN and leptophos, respectively. Sheepshead minnows were relatively insensitive to leptophos with a 96-h LC50 of 188.9 µg/l.

Survivors of the 96-h toxicity tests were analyzed for EPN and leptophos residues. The test animals and their average bioconcentration factors for EPN after a 96-h exposure were: pink shrimp, non-detectable (< 0.02 mg/kg); spot 260 ×; and pinfish, 774 ×. Average bioconcentration factors for pink shrimp and spot exposed to leptophos for 96 hours were 530 × and 222 ×, respectively.

Mysid Entire Life-cycle Studies

Results of entire life-cycle exposures of mysids to EPN and leptophos are shown in Tables 2 and 3. These data show that both insecticides exhibited toxic

TABLE 2.

Toxicity of EPN and leptophos to *Mysidopsis bah'a* in two life cycle exposures. Test duration for the EPN study was 28 days; for leptophos, 21 days.

INSECTICIDE	MEASURED CONCENTRATION (µg/ℓ)	%MORTALITY	NO. YOUNG/FEMALE
EPN	0.0	10	16.1
	0.0	3	14.6
	0.067	0	18.5
	0.076	8	14.8
	0.44	8	15.9
	4.13	68*	3.5*
LEPTOPHOS	0.0	12	5.1
	0.64	24	7.4
	1.77	24	2.2*
	3.63	54*	0*
	5.63	74*	0*

*Significantly different from control at $\alpha = 0.05$.

TABLE 3.

Summary of acute and entire life-cycle exposure of *Mysidopsis bahia* to EPN and leptophos.

INSECTICIDE	96-hr LC50 (95% Confidence Interval)	MATC*, µg/ℓ (Geometric Mean)	A.F.** (Geometric Mean)
EPN	3.44 (2.49 - 5.84)	0.44 - 4.13 (1.35)	0.13 - 1.2 (0.40)
LEPTOPHOS	3.16 (1.89 - 5.67)	0.64 - 1.77 (1.06)	0.20 - 0.56 (0.33)

*MATC = Maximum Acceptable Toxicant Concentration; Mount and Stephan 1967.
**A.F. = Application Factor; the ratio of the MATC to the 96-hr LC50.

effects on mysids at low concentrations. In the EPN study, mysids exposed to 4.13 µg/l suffered significantly greater ($\alpha = 0.05$) mortality and produced fewer young per female than the control mysids (Table 2). In the leptophos study mysids exposed to concentrations $\geq$ 3.63 µg/l suffered significant mortality and in the 1.77 µg/l concentration produced fewer young per female than the control mysids. The geometric mean of the Maximum Acceptable Toxicant Concentration (MATC, Mount and Stephan, 1967) and the lowest concentration producing adverse effects with both EPN and leptophos are approximately 0.3 × their respective 96-h LC50 (Table 3). Therefore, the acute toxicity of EPN and leptophos to mysids is a relatively close estimate of chronic toxicity.

Bioconcentration Studies

Pinfish exposed to 2.4 µg/l EPN (measured concentration) bioconcentrated the insecticide rapidly and reached an apparent equilibrium in approximately

48 h (Fig. 1). Uptake/depuration data for the 0.17 μg/l concentration (0.25 μg/l, nominal concentration) were not illustrated because residues in these tissues were usually below the level of detectability (0.02 mg/kg). At equilibrium (plateau) the concentration of EPN in whole pinfish was approximately 1.7 mg/kg giving a bioconcentration factor (BCF; concentration of EPN in fish tissues divided by the measured concentration of EPN in exposure water) of 707 X. By comparison, pinfish exposed to EPN for 96 h in the acute study, bioconcentrated an average of 744 X.

After a four-day post exposure period, only one pinfish of four sampled had detectable (0.041 mg/kg) EPN in its tissues; after eight days, no residues were detected in any fish sampled.

Spot exposed to 0.61 μg/l measured concentrations (0.4 μg/l nominal) of leptophos bioconcentrated the insecticide to an apparent equilibrium of 0.04 mg/kg in their remaining tissues (Fig. 2). No residues were found in edible tissues above the detectable limit (0.025 mg/kg). In addition, no detectable residues were found in fish exposed to 0.04 μg leptophos/litre seawater. The BCF of leptophos at equilibrium for spot was 68 ×. After a 4-day post exposure period, no leptophos was detected in tissues. By comparison, spot exposed to leptophos for 96 h in the acute study bioconcentrated an average of 22 ×. The higher average BCF in the acute study appears to conflict with the results of the 26-day study. However, the BCF at 0.78 μg/l concentration (the exposure concentration in the acute test closest to the 26-day study, 0.6 μg/l) was 86 ×. In general, the BCF appeared to be directly related to exposure concentration.

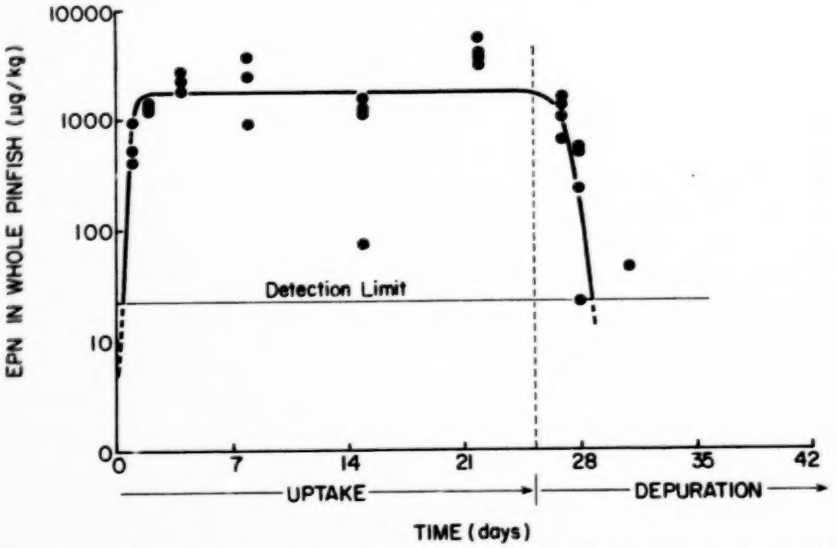

FIG. 1. Uptake and depuration of EPN in whole-body tissues of pinfish (*Lagodon rhomboides*) in a flowering seawater bioassay. Exposure concentration average 2.4 μg=l EPN per liter seawater.

DISCUSSION

Our 96-h EPN acute toxicity test results reported are comparable to those reported for freshwater species. Sanders (1972) reported 96-h LC50 values of 6.8 and 0.56 μg/l for scud (*Gammarus fasciatus*) and glass shrimp (*Palaemonetes kadiakensis*), respectively. Pickering *et al.* (1962) reported the 96-h LC50 values of EPN for several freshwater fishes: bluegill (*Lepomis macrochirus*), 100 μg/l; fathead minnows (*Pimphales promelas*), 250 μg/l; goldfish (*Carassius amatus*), 450 μg/l; and guppies (*Lebistes reticulatus*), 32 μg/l. Korn and Earnest (1974) exposed juvenile striped bass (*Morone saxatilis*) to EPN in flowing seawater and calculated a 96-hour LC50 of 60 μg/l. Our data on spot (LC50 = 25.6 μg/l) and pinfish (18.3 μg/l) show that these species are more sensitive than freshwater fishes; sensitivity of sheepshead minnows to EPN (LC50 = 189 μg/l) was comparable to those of fathead minnows and bluegills.

The data from the pink shrimp exposure to EPN are cause for particular concern. Shrimp were especially sensitive to EPN (LC50 = 0.29 μg/l), and there was even 20% mortality among shrimp exposed for 96 hours to a non-detectable concentration (0.032 μg/l, nominal). Further, since no EPN was detected in tissues of shrimp exposed to concentrations as high as 0.42 μg/l, it is conceivable that pink shrimp in estuarine habitats could suffer significant mortality if exposed to EPN for greater than 96 hours to concentrations below detection limits (0.02 μg/l) and the identity of the toxicant causing the mortality could not be determined by residue analysis.

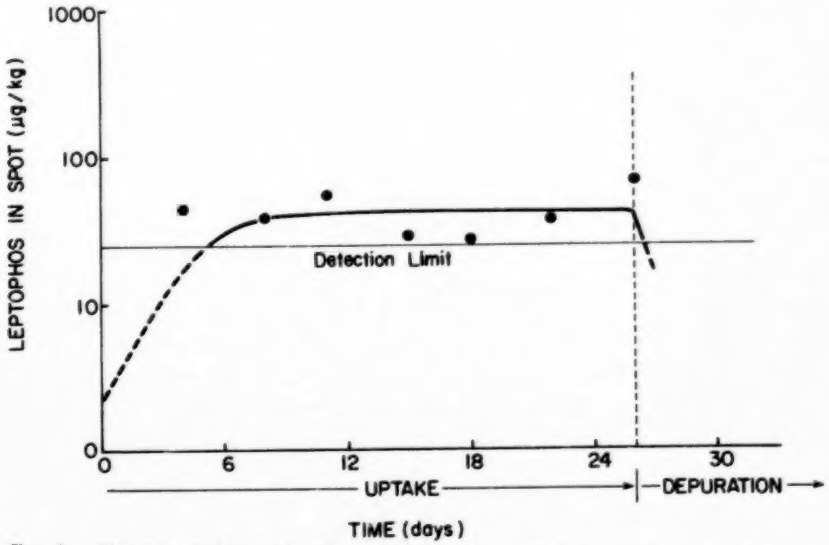

FIG. 2. Uptake and depuration of leptophos in tissues of spot (*Leiostomus xanthurus*) in a flowing seawater bioassay. Exposure concentration averaged 0.61 μg leptophos per litre seawater.

We found no leptophos acute toxicity data on fishes or aquatic invertebrates in the literature which could be compared with our values.

Results of the mysid chronic studies with EPN and leptophos indicate that the geometric means of the MATC's for each insecticide were 1.35 and 1.06 μg-l, respectively, giving application factors of 0.40 for EPN and 0.33 for leptophos. We are unaware of any published data which describe the chronic toxicity of EPN or leptophos to any other estuarine species. However, a leptophos 28-day embryo/larval study was conducted on sheepshead minnows. No significant effects ($\alpha = 0.05$) on survival were noted in fish exposed to concentrations as high as 15 μg/l; however, significant effects on growth occurred in 28 days (Larry R. Goodman, Environmental Research Laboratory, Gulf Breeze, Florida; personal communication). Results of the mysid studies and the sheepshead minnows embryo/larval studies indicate that chronic effects are not anticipated at concentrations much less than 0.3 × the 96-hr LC50. By comparison, Kepone® (an organochlorine insecticide) produced deleterious effects in sheepshead minnow embryo/larval study indicate that chronic effects are not anticipated at reproduction in mysids at 0.04 × the 96-h LC50 of 10.1 μg/l (Nimmo *et al.* 1977).

From our studies, the bioconcentration factors (BCF) and depuration rates of EPN and leptophos indicate that the insecticides would not accumulate to high concentrations in the aquatic food web. In the event of aquatic contamination, both insecticides should depurate quickly. Our data on leptophos appear to conflict with those of Metcalf and Sanborn (1975) who studied the insecticide in a terrestrial-aquatic model ecosystem. Their aquatic system was dosed with a single application of ^{14}C-labeled leptophos and the distribution of the insecticide was monitored at the end of 45 days. Bioconcentration factors for the plants and animals were based on measured concentrations of leptophos in water at the end of the test. If the pesticide in water disappeared with time, we feel that concentrations at 45 days may overestimate the bioconcentration factors. Therefore, no real comparison of our data and those of Metcalf and Sanborn (1975) can be made.

Bioconcentration factors of 707 for EPN and 68 for leptophos which we determined in our study are far below those for fishes exposed to most organochlorine insecticides. For example, endosulfan was bioconcentrated by mullet 2755 × in 28 days (Schimmel *et al.* 1977a); heptachlor was bioconcentrated by spot 3,600 × and trans-chlordane 4,600 × in 24 days (Schimmel *et al.* 1976). However, the bioconcentration factor for BHC in pinfish (180 × in edible tissues, 617 × in offal; Schimmel *et al.* 1977b) was similar to those reported in this paper.

In summary, both EPN and leptophos were acutely toxic to both crustaceans and fishes, the commercial pink shrimp was the most sensitive species tested with 96-h LC50 values of 0.29 μg/l for EPN and 1.88 μg/l for leptophos. In chronic studies with mysid shrimp, both EPN and leptophos exhibited adverse effects at concentrations of approximately 0.3 × the 96-h LC50 indicating that acute toxicity is a relatively close estimate of chronic toxicity. In two bioconcen-

tration studies, EPN and leptophos were taken up rapidly by two estuarine fishes to equilibrium in two to four days with bioconcentration factors of 707 × and 68 ×, respectively. After eight days in EPN-free seawater, no EPN was measured in pinfish tissues; no leptophos was measured in spot tissues after four days in leptophos-free seawater. Our data indicate that if EPN or leptophos contaminated estuarine environments in concentrations ≥ 1.0 μg/l, the most deleterious effect on crustaceans and possibly fishes would result from acute toxicity, not chronic toxicity or bioconcentration of the insecticides.

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